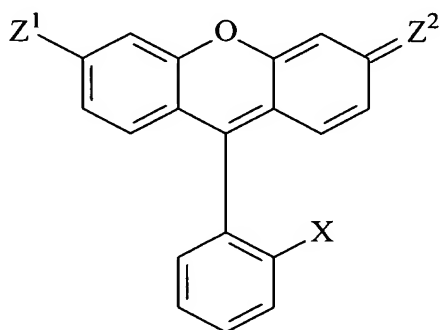


## CLAIMS

We claim:

- 5           1.       An energy-transfer dye comprising:  
            a donor dye capable of absorbing light at a first wavelength and emitting  
            excitation energy in response thereto;  
            an acceptor dye capable of absorbing the excitation energy emitted by the  
            donor dye and fluorescing at a second wavelength in response; and  
10           a linker for linking the donor dye and the acceptor dye;  
            wherein at least one of the donor dye and acceptor dye is an atropisomerically  
            enriched xanthene compound having the structure:



- Z<sup>1</sup> is OH, NH<sub>2</sub>, NHR, or NR<sub>2</sub>, wherein each R is independently hydrogen,  
15           C<sub>1</sub>–C<sub>12</sub> alkyl, phenyl, benzyl, aryl, heterocycle, or a linking moiety;  
            Z<sup>2</sup> is O, <sup>+</sup>NH<sub>2</sub>, <sup>+</sup>NHR, or <sup>+</sup>NR<sub>2</sub>, wherein each R is independently hydrogen,  
            C<sub>1</sub>–C<sub>12</sub> alkyl, phenyl, benzyl, aryl, heterocycle, or a linking moiety;  
            X is carboxylate or sulfonate;  
            and said structure includes aryl-substituted forms thereof.  
20           2.       The energy-transfer dye of claim 1 wherein the xanthene compound is a  
            substantially pure atropisomer.  
  
            3.       The energy-transfer dye of claim 1 wherein the donor dye is an enriched  
            atropisomer of a xanthene compound and the acceptor dye is a cyanine, a  
            phthalocyanine, a squaraine, a bodipy, a benzophenoxazine, a fluorescein, a  
25           dibenzorhodamine, or a rhodamine dye.

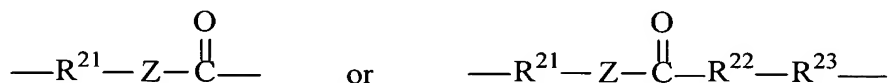
4. The energy-transfer dye of claim 1 wherein the acceptor dye is an enriched atropisomer of a xanthene compound and the donor dye is linked to the xanthene compound and to a polynucleotide.

5. The energy-transfer dye of claim 3 wherein the donor dye is linked to the 5'-terminus of the polynucleotide.

6. The energy-transfer dye of claim 3 wherein the donor dye is linked to the 3'-terminus of the polynucleotide.

7. The energy-transfer dye of claim 3 wherein the donor dye is linked to a nucleobase of the polynucleotide, wherein if the nucleobase is a purine, the linker is attached at the 8-position, if the nucleobase is a 7-deazapurine, the linker is attached at the 7-position or 8-position, and if the nucleobase is a pyrimidine, the linker is attached at the 5-position.

8. The energy-transfer dye of claim 1 wherein the linker has the structures:



15 wherein

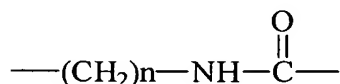
Z is selected from the group consisting of NH, S and O;

R<sup>21</sup> is a C<sub>1</sub>–C<sub>12</sub> alkyl attached to the donor dye;

R<sup>22</sup> is a substituent selected from the group consisting of a C<sub>1</sub>–C<sub>12</sub> alkylidyl, a five and six membered ring having at least one unsaturated bond and a fused ring structure which is attached to the carbonyl carbon; and

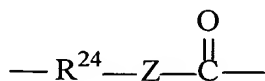
R<sup>23</sup> includes a functional group which attaches the linker to the acceptor dye.

9. The energy-transfer dye of claim 8 wherein the linker has the structure:



and n ranges from 2 to 10.

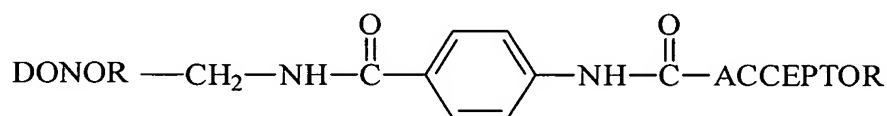
25 10. The energy-transfer dye of claim 8 wherein R<sup>23</sup> has the structure



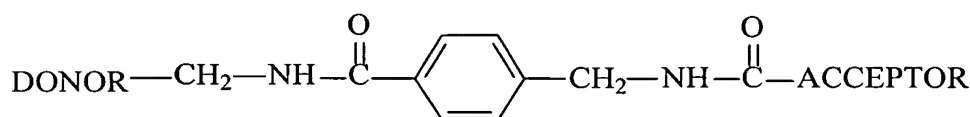
wherein R<sup>24</sup> is a C<sub>1</sub>-C<sub>12</sub> alkyl.

11. The energy-transfer dye of claim 8 wherein R<sup>22</sup> is a five or six membered ring selected from the group consisting of cyclopentene, cyclohexene, cyclopentadiene, cyclohexadiene, furan, thiofuran, pyrrole, isopyrrole, isoazole, pyrazole, isoimidazole, pyran, pyrone, benzene, pyridine, pyridazine, pyrimidine, pyrazine oxazine, indene, benzofuran, thionaphthene, indole and naphthalene.

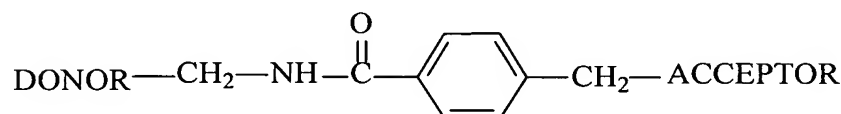
12. The energy-transfer dye of claim 1 wherein the linker has the structure



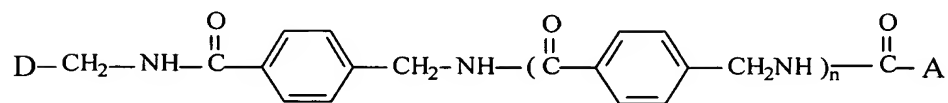
13. The energy-transfer dye of claim 1 wherein the linker has the structure



14. The energy-transfer dye of claim 1 wherein the linker has the structure

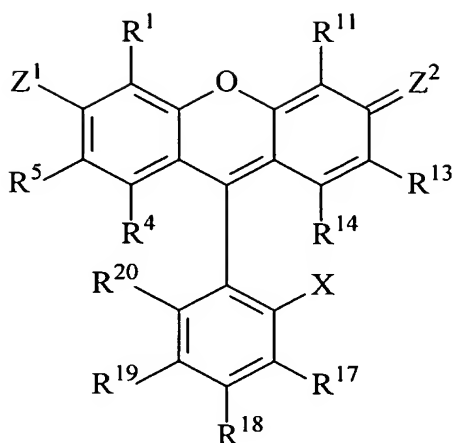


15. The energy-transfer dye of claim 1 in which the linker has the structure:



wherein D is a donor dye, A is an acceptor dye and n is 1 or 2.

16. The energy-transfer dye of claim 1 wherein the linker is attached at R<sup>1</sup>, R<sup>11</sup>, R<sup>18</sup> or R<sup>19</sup> of the xanthene compound having the structure:



17. The energy-transfer dye of claim 16 wherein a linker to the donor or the acceptor is attached to R<sup>1</sup> or R<sup>11</sup>, and a linker to a polynucleotide is attached to R<sup>18</sup> or R<sup>19</sup>.

18. The energy-transfer dye of claim 16 wherein the linker is attached at one of positions Z<sup>1</sup> or Z<sup>2</sup> of the substantially pure atropisomeric xanthene compound.

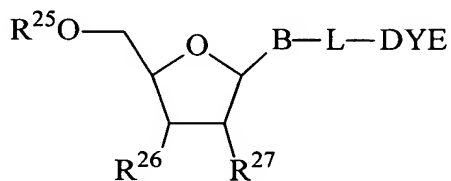
19. The energy-transfer dye of claim 1 wherein a linking moiety is selected from azido, monosubstituted primary amine, disubstituted secondary amine, thiol, hydroxyl, halide, epoxide, N-hydroxysuccinimidyl ester, carboxyl, isothiocyanate, sulfonyl chloride, sulfonate ester, silyl halide, chlorotriazinyl, succinimidyl ester, pentafluorophenyl ester, maleimide, haloacetyl, epoxide, alkylhalide, allyl halide, aldehyde, ketone, acylazide, anhydride, and iodoacetamide.

20. The energy-transfer dye of claim 1 wherein an aryl substituent is independently selected from fluorine, chlorine, C<sub>1</sub>–C<sub>8</sub> alkyl, carboxylate, sulfate, sulfonate (–SO<sub>3</sub><sup>–</sup>), alkylsulfonate (–R–SO<sub>3</sub><sup>–</sup>), aminomethyl (–CH<sub>2</sub>NH<sub>2</sub>), aminoalkyl, 4-dialkylaminopyridinium, hydroxymethyl (–CH<sub>2</sub>OH), methoxy (–OCH<sub>3</sub>), hydroxyalkyl (–ROH), thiomethyl (–CH<sub>2</sub>SH), thioalkyl (–RSH), alkylsulfone (–SO<sub>2</sub>R), arylthio (–SAr), arylsulfone (–SO<sub>2</sub>Ar), sulfonamide (–SO<sub>2</sub>NR<sub>2</sub>), alkylsulfoxide (–SOR), arylsulfoxide (–SOAr), amino (–NH<sub>2</sub>), ammonium (–NH<sub>3</sub><sup>+</sup>), amido (–CONR<sub>2</sub>), nitrile (–CN), C<sub>1</sub>–C<sub>8</sub> alkoxy (–OR), phenoxy, phenolic, tolyl, phenyl, aryl, benzyl, heterocycle, phosphonate, phosphate, quaternary amine, sulfate, polyethyleneoxy, and linking moiety.

21. The energy-transfer dye of claim 20 wherein a heterocycle is selected

from 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 2-imidazole, 4-imidazole, 3-pyrazole, 4-pyrazole, pyridazine, pyrimidine, pyrazine, cinnoline, pthalazine, quinazoline, quinoxaline, 3-(1,2,4-*N*-triazolyl, 5-(1,2,4-*N*-triazolyl, 5-tetrazolyl, 4-(1-*O*, 3-*N*)-oxazole, 5-(1-*O*, 3-*N*)-oxazole, 4-(1-*S*, 3-*N*)-thiazole, 5-(1-*S*, 3-*N*)-thiazole, 2-benzoxazole, 2-benzothiazole, 4-(1,2,3-*N*-benzotriazole, and benzimidazole.

22. A labelled nucleoside or nucleotide having the formula:



wherein DYE is the energy-transfer dye of claim 1;

10 B is a nucleobase;

L is a linker;

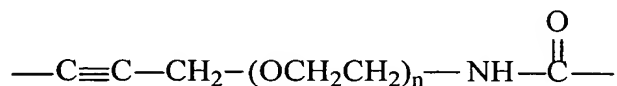
$R^{25}$  is H, monophosphate, diphosphate, triphosphate, thiophosphate, or phosphate analog; and

15  $R^{26}$  and  $R^{27}$ , when taken alone, are each independently H, HO, F, or a moiety which blocks polymerase-mediated target-directed polymerization, or when taken together form 2'-3'-didehydroribose.

23. The labelled nucleoside or nucleotide of claim 22 wherein the xanthene compound of the energy-transfer dye is a substantially pure atropisomer.

20 24. The labelled nucleoside or nucleotide of claim 22 wherein B is selected from the group consisting of uracil, thymine, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanosine.

25. The labelled nucleoside or nucleotide of claim 22 in which L is:



wherein n is 0, 1, or 2.

25 26. The labelled nucleoside or nucleotide of claim 22 which is enzymatically incorporatable.

27. The labelled nucleoside or nucleotide of claim 22 which is a terminator.

28. The labelled nucleoside or nucleotide of claim 27 wherein  $R^{26}$  and  $R^{27}$ , when taken alone, are each independently H, F, or a moiety which blocks polymerase-mediated target-directed polymerization, or when taken together form 2'-3'-

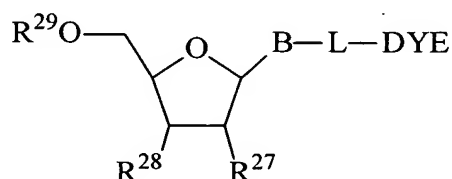
5 didehydroribose.

29. The labelled nucleoside or nucleotide of claim 22 which is enzymatically extendable.

30. A labelled polynucleotide comprising a polynucleotide covalently attached to the energy-transfer dye of claim 1.

10 31. The labelled polynucleotide of claim 30 wherein the xanthene compound of the energy-transfer dye is a substantially pure atropisomer.

32. The labelled polynucleotide of claim 30 comprising the formula:



wherein DYE is the energy-transfer dye;

15 B is a nucleobase;

L is a linker;

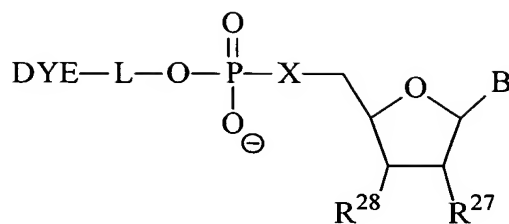
$R^{27}$  is H, OH, halide, azide, amine, alkylamine,  $C_1$ - $C_6$  alkyl, allyl,  $C_1$ - $C_6$  alkoxy,  $OCH_3$ , or  $OCH_2CH=CH_2$ ; and

20  $R^{28}$  and  $R^{29}$  when taken alone, are each independently H, phosphate, internucleotide phosphodiester, or internucleotide analog;

wherein the polynucleotide comprises 2 to 100 nucleotides.

33. The labelled polynucleotide of claim 32 wherein B is selected from the group consisting of uracil, thymine, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanosine.

25 34. The labelled polynucleotide of claim 30 comprising the formula:



wherein DYE is the energy-transfer dye;

B is a nucleobase;

X is O, NH, or S;

5 L is a linker;

R<sup>27</sup> is H, OH, halide, azide, amine, alkylamine, C<sub>1</sub>-C<sub>6</sub> alkyl, allyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, OCH<sub>3</sub>, or OCH<sub>2</sub>CH=CH<sub>2</sub>; and

R<sup>28</sup> is internucleotide phosphodiester or internucleotide analog;

wherein the polynucleotide comprises 2 to 100 nucleotides.

10 35. The labelled polynucleotide of claim 34 wherein B is selected from the group consisting of uracil, thymine, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanosine.

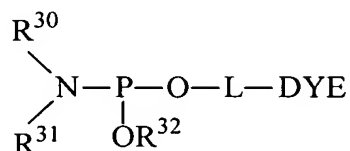
36. The labelled polynucleotide of claim 34 in which L is C<sub>1</sub>-C<sub>12</sub> alkylidyl.

15 37. The labelled polynucleotide of claim 34 in which L comprises -(CH<sub>2</sub>CH<sub>2</sub>O)<sub>n</sub>-, where n is 1 to 100.

38. A labelled polypeptide comprising a polypeptide covalently attached to an energy-transfer dye of claim 1.

39. The labelled polypeptide of claim 38 wherein the xanthene compound of the energy-transfer dye is a substantially pure atropisomer.

20 40. A phosphoramidite compound having the formula:



wherein DYE is the energy-transfer dye of claim 1;

L is a linker;

$R^{30}$  and  $R^{31}$  taken separately are selected from the group consisting of  $C_1$ - $C_{12}$  alkyl,  $C_1$ - $C_{12}$  cycloalkyl, and aryl; or  $R^{30}$  and  $R^{31}$  taken together with the nitrogen atom form a saturated nitrogen heterocycle; and

$R^{32}$  is a phosphite ester protecting group.

5            41.     The phosphoramidite compound of claim 40 wherein the xanthene compound of the energy-transfer dye is a substantially pure atropisomer.

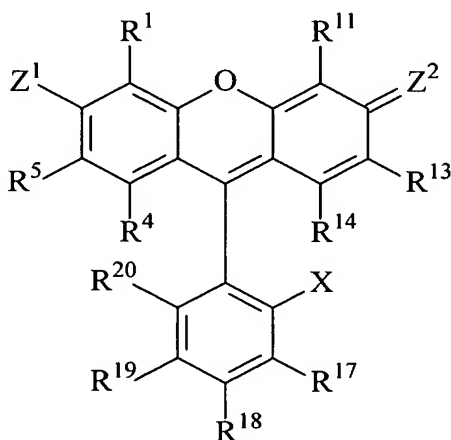
              42.     The phosphoramidite compound of claim 40 wherein  $R^{32}$  is selected from the group consisting of methyl, 2-cyanoethyl, and 2-(4-nitrophenyl)ethyl.

10           43.     The phosphoramidite compound of claim 40 wherein  $R^{30}$  and  $R^{31}$  are each isopropyl.

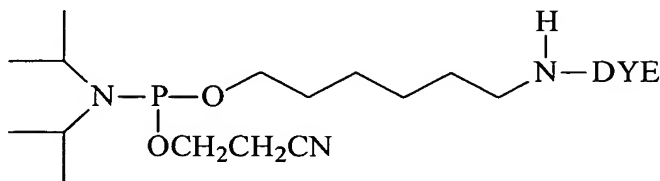
              44.     The phosphoramidite compound of claim 40 wherein  $R^{30}$  and  $R^{31}$  taken together is morpholino.

              45.     The phosphoramidite compound of claim 40 wherein L is  $C_1$ - $C_{12}$  alkyldiyl.

15           46.     The phosphoramidite compound of claim 40 wherein L is attached at  $R^{18}$  or  $R^{19}$  of DYE having the structure:

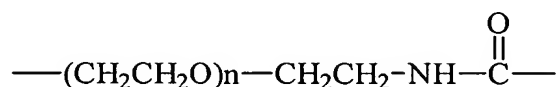


47.     The phosphoramidite compound of claim 46 having the structure:





48. The phosphoramidite compound of claim 40 wherein L is



and n ranges from 1 to 10.

49. A kit for labelling a polynucleotide, comprising an energy-transfer dye  
5 according to claim 1 and a polynucleotide.

50. A kit for labelling a polynucleotide, comprising a phosphoramidite compound  
according to claim 40 and a polynucleotide.

51. A kit for labelling a polypeptide, comprising an energy-transfer dye according  
to claim 38 and a polypeptide.

10 52. A kit for generating a labelled primer extension product, comprising one or  
more enzymatically-incorporatable nucleotides and a primer, wherein said primer is a  
labelled polynucleotide according to claim 30.

53. A kit for generating a labelled primer extension product, comprising one or  
more enzymatically-incorporatable nucleotides and a primer, wherein at least one  
15 nucleotide is a labelled nucleotide according to claim 22.

54. The kit of claim 53 wherein the labelled nucleotide is a terminator.

55. The kit of claim 54 which comprises four different terminators, one which  
terminates at a target A, one which terminates at a target G, one which terminates at a  
target C and one which terminates at a target T or U.

20